

Seryl-tRNA in Mammalian Tissues: Chromatographic Differences in Brain and Liver and a Specific Response to the Codon, UGA

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Communicated by Marshall Nirenberg, August 31, 1970

Abstract. Differences in the elution profiles of seryl-tRNAs of beef and of rabbit liver and brain were observed. A species of beef seryl-tRNA obtained by reversed phase column chromatography that responds specifically to the codon UGA was found. In addition, a species of rabbit and of chicken seryl-tRNA that recognizes UGA was found.

Chromatographic differences in tRNA of higher organisms have been reported in response to hormone treatment,¹⁻³ in differentiation,^{3,4} in oncogenesis,⁵⁻⁹ in similar cells making different proteins,^{10,11} and in organs and cells grown in culture.^{12,13} Codon responses to fractionated tRNA in higher organisms have been reported.^{14,15} The purpose of the present investigation was to study seryl-tRNA in mammalian tissues and to examine codon responses of individual species.

Materials and Methods. Transfer RNA was prepared from beef and from rabbit brain and liver as follows: Each tissue was homogenized in 3.5 volumes of a solution (Solution A) that contained 0.05 M Tris-HCl buffer, pH 7.4; 0.35 M sucrose; 0.04 M MgCl₂; 0.025 M KCl; 0.035 M KHCO₃; 5 mM mercaptoethanol; the particulate cell fractions were removed according to Yang and Novelli.¹⁶ An equal volume of water-saturated phenol was then added, and the mixture shaken for 20 min at 24°C. After separation of the aqueous and phenol layers by centrifugation, the aqueous layer was aspirated and treated once more with phenol as above and, finally, with an equal volume of CHCl₃. The original phenol layer was extracted with an equal volume of Solution A; after separation, the aqueous layer was used to extract the second phenol and the CHCl₃ layers. The aqueous fractions were pooled and 0.1 volume of 20% potassium acetate and 2.5 volumes of 95% ethanol were added. This solution was left at -20°C overnight. The precipitate was collected and dissolved in a minimum volume of 1.8 M Tris-HCl buffer, pH 8.0. The Tris-HCl solution was left at 37°C for 1 hr to discharge amino acids from tRNA.¹⁷ After deacylation, 2.5 volumes of ethanol were added, and the mixture was left at -20°C for a minimum of 4 hr. The resulting precipitate was collected, redissolved in water, lyophilized, and stored at -20°C. Transfer RNA was also prepared from chicken liver by this method.

Amino acyl-tRNA synthetases were prepared from each tissue as follows: Fresh tissues were minced, then homogenized in 3.5 volumes of a solution (Solution B) containing 0.05 M Tris-HCl buffer, pH 7.4; 0.35 M sucrose; 0.05 M MgCl₂; 0.0025 M KCl; 0.035 M KHCO₃; and 0.5 mM dithiothreitol (Cleland's reagent). The heavy debris was removed by centrifugation at 20,000 × *g*. After centrifugation at 105,000 × *g* for 45 min, the extracts were adjusted to 0.1 M with respect to Tris-HCl buffer, pH 7.4, and NaCl. The endogenous tRNA and microsomal fragments were then removed by mix-

ing each extract with an equal volume of DEAE-cellulose, previously equilibrated with Solution B (adjusted to 0.1 M with respect to Tris-HCl buffer, pH 7.4, and NaCl), stirring for 15 min, and subsequently removing the DEAE-cellulose. Extracts were then brought to 75% saturation with $(\text{NH}_4)_2\text{SO}_4$. The resulting precipitates were collected, dissolved in one-fourth of the original volume of Solution B, and stored in liquid nitrogen.

Reaction mixtures for seryl-tRNA formation contained: ATP, 6 mM; Tris-HCl buffer, pH 7.4, 120 mM; MgCl_2 , 20 mM; 19-L- ^{14}C amino acids, 4.4 mM; radioactive serine; tRNA; and aa-tRNA synthetases. Optimal conditions for seryl-tRNA formation (radioactive serine and enzymes in excess and tRNA limiting) were determined for all tRNA preparations. Reaction mixtures were incubated at 37°C. Control reactions demonstrated that 1 mM puromycin had no effect on charging and that addition of 1 M KOH at the end of the reaction completely removed trichloroacetic acid-precipitable counts. After removal of protein with phenol, and subsequent removal of free amino acids, ATP, and phenol by passage through a Sephadex G-25 column, large-scale preparations were applied to the reversed-phase chromatographic system of Kelmers *et al.*¹⁸ Radioactive chemicals were obtained from New England Nuclear Corp.

Results. Chromatography of seryl-tRNA: Chromatography of seryl-tRNA from beef brain and liver is shown in Fig. 1. Very marked differences were ob-

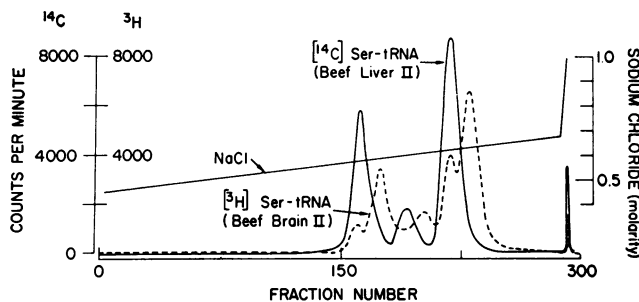


FIG. 1. Comparison of elution profiles of seryl-tRNA of beef liver and brain. Liver tRNA was acylated with liver aa-tRNA synthetases and ^{14}C serine (—) and compared with brain tRNA acylated with brain aa-tRNA synthetases and ^3H serine (---). Columns (1 × 240 cm) were prepared according to Kelmers *et al.*¹⁸ and used at 24°C. Three-liter gradients were employed at a flow rate of 2.5 ml/min. 10 ml fractions were collected. Radioactivity was detected in eluates by precipitating seryl-tRNA in 10% trichloroacetic acid, collecting precipitates on nitrocellulose filters (0.45 μm), and counting in a Packard liquid scintillation counter. Specific radioactivities of ^{14}C - and ^3H serine are 123 mCi/mmol and 3730 mCi/mmol, respectively.

served in the elution profiles. There were four major peaks of seryl-tRNA in liver and six in brain. Seryl-tRNA of beef liver was also compared with that of beef kidney and virtually identical profiles were observed.

The differences observed in beef liver and brain seryl-tRNA were also observed when: (1) tRNA was prepared from homogenates of unfractionated tissue; (2) tRNA of brain was acylated with ^{14}C serine and liver with ^3H serine; and (3) tRNA of brain was acylated with liver enzyme and liver tRNA with brain enzyme. These studies demonstrated that the differences observed were present in the isolated tRNA of each tissue and were not due to the tRNA of a cellular component (e.g., mitochondria^{19,20}), to isotope, or to a tissue-specific seryl-tRNA synthetase.

Although the above studies demonstrate that the observed chromatographic differences are present in isolated tRNA, they do not rule out the possibility that these differences may be due to changes occurring during isolation. Studies from other laboratories have shown that tRNA may be modified by: (1) action of caustic reagents used in tRNA preparation;²¹ (2) dimer formation;^{22,23} (3) loss of CCA terminus;²⁴ and (4) treatment with magnesium, which may activate an inactive species of tRNA.²⁵ The differences observed between seryl-tRNA of beef brain and liver do not appear to be due to these four factors or to a tissue specific nuclease, as will be reported elsewhere.

Transfer RNA was prepared from rabbit brain and liver to determine if similar differences in seryl-tRNA are present in another mammalian species. Similar differences as those observed in beef liver and brain were also observed in these rabbit tissues (Fig. 2).

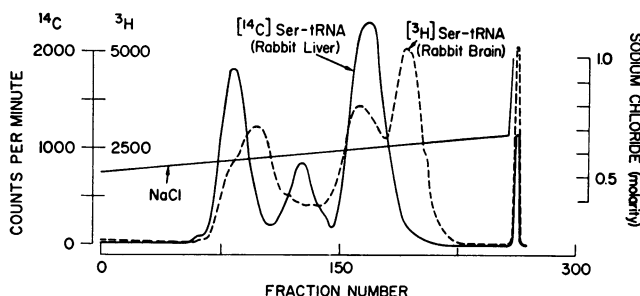


FIG. 2. Comparison of elution profiles of seryl-tRNA of rabbit liver and brain. Liver tRNA was acylated with rabbit liver aa-tRNA synthetases and [¹⁴C]serine (—) and compared to brain tRNA acylated with rabbit brain aa-tRNA synthetases and [³H]serine (---). Column conditions, detection of radioactivity, and specific radioactivities of serine were the same as those in Fig. 1.

Codon recognition studies of beef seryl-tRNA: Seryl-tRNA of beef brain and liver was prepared for codon recognition studies by reversed-phase chromatography (Fig. 3A and B). Peaks I, III, and IV in liver correspond to I, IV, and VI, respectively, in brain; II and V in brain constitute peaks that are not evident in liver (see Fig. 1).

Each peak in liver and brain was assayed with trinucleoside diphosphates using the known serine codons, UCU, UCC, UCA, UCG, AGU, and AGC,^{26,27} and with the codons, UGA, UAA, and UAG, which are known to be involved in the termination of protein synthesis in bacteria.^{28,29} In each assay 0.01 M Mg⁺⁺ was employed to minimize the possibility of ambiguous codon recognition by aa-tRNA.^{14,26} The results of these studies are given in Table 1. Peak I of liver recognized AGU and AGC; Peak II, UCG; and Peak III, UCU, UCC, and UCA (Table 1). Peak IV of liver, which eluted with 1 M sodium chloride (Fig. 3A), recognized UGA. There was also a slight response of Peak IV to UCU, but this probably was due to the presence of a small amount of the seryl-tRNA which recognizes UCU (see below). The codon responses of Peaks I–III were similar to those reported by Caskey *et al.*¹⁴ for guinea pig liver seryl-tRNA. A response of guinea pig liver cysteinyl-tRNA to UGA has been observed;^{14,30} however, this

TABLE 1. Codon responses of fractioned liver and brain seryl-tRNA.

Codon	Fraction											
	Liver				Brain							
	I	II	III		IV	I	II	III		IV	V	VI
			Δ pmol bound*					Δ pmol bound*				
UGA	-0.012	0.001	0.008	0.159	-0.013	-0.021	-0.005	-0.047	-0.011	0.279		
UCU	-0.004	0.013	1.022	0.015	-0.004	-0.024	0.022	0.450	0.439	0.176		
UCC	-0.007	0.007	0.030	0.010	-0.014	-0.023	-0.001	0.007	0.026	0.012		
UCA	-0.002	0.007	0.390	0.003	-0.001	-0.010	0.007	0.160	0.147	0.054		
UCG	0.002	0.679	0.009	-0.006	0.004	-0.008	0.719	0.006	-0.001	-0.007		
AGU	0.130	0.022	-0.013	-0.004	0.629	0.493	0.005	-0.043	-0.005	-0.020		
AGC	0.164	0.021	-0.014	-0.009	0.520	0.554	0.005	-0.048	-0.002	-0.008		
UAA	-0.009	-0.001	-0.018	-0.004	-0.010	-0.029	-0.005	-0.038	-0.009	-0.010		
UAG	-0.008	0	-0.010	0	-0.009	0.013	-0.005	-0.046	-0.005	-0.014		
None†	(0.043)	(0.022)	(0.131)	(0.046)	(0.057)	(0.077)	(0.027)	(0.111)	(0.045)	(0.059)		
A_{260} units tRNA	0.33	0.06	0.01	0.32	0.12	0.04	0.02	0.02	0.01	0.02		
pmol Ser-tRNA	0.873	1.534	2.770	0.820	1.521	3.298	1.325	3.140	1.585	1.727		

Codon responses of fractioned liver and brain seryl-tRNA. Assay conditions were the same as those of Nirenberg and Leder,²⁷ except that 0.01 M magnesium acetate and 0.05 M Tris-acetate buffer, pH 7.2, were employed. Reaction mixtures contained 2.48 A_{260} units of *E. coli* ribosomes (from Dr. R. Tompkins) and between 0.15 and 0.20 A_{260} units of trinucleotide.

* Amount of seryl-tRNA bound to ribosomes in presence of codon minus the amount bound in absence of codon.

† Amount of seryl-tRNA bound to ribosomes in absence of codon.

TABLE 2. Codon responses of Peak VI of brain seryl-tRNA.

Codons and pmol of seryl-tRNA bound												
Codon:	UGA	UCU	UCA	UGU	UGC	UGG	UUA	AUG	AGA	CGA	GGA	None*
Δ^\dagger	0.207	0.002	-0.004	-0.001	0	-0.005	0	-0.012	-0.005	-0.003	-0.011	(0.040)

Codon responses of Peak VI of brain seryl-tRNA. Assay conditions as in Table 1. 1.242 pmol of [³H]seryl-tRNA (0.06 A_{260} units) were added to each reaction mixture. Brain seryl-tRNA was fractionated using 0.4-0.8 M sodium chloride to provide greater separation between Peaks V and VI. Only Peak VI was assayed.

* Amount of seryl-tRNA bound to ribosomes in absence of codon.

† Amount of seryl-tRNA bound to ribosomes in presence of codon minus the amount bound in absence of codon.

response was reported to be ambiguous, since fractionated cysteinyl-tRNA of guinea pig liver recognized UGA, UGU, and UGC at 0.02 M Mg^{++} , but only UGU and UGC at 0.01 M Mg^{++} (ref. 14).

Peaks I and II of brain recognized AGU and AGC; Peak III, UCG; and Peaks IV and V, UCU, UCC, and UCA (Table 1). Under these conditions of fractionation, Peak VI of brain responded to both UGA and UCU. Brain seryl-tRNA was fractionated with a steeper salt gradient (Fig. 3C) to determine if the UGA and UCU response could be resolved. The steeper salt gradient gave greater separation between Peaks V and VI. Under these conditions, Peak VI responded to UGA, but not to UCU (Table 2). Furthermore, Peak VI did not respond to AUG, which is involved in initiation of protein biosynthesis in microorganisms and possibly in mammalian systems^{31,32} (Table 2). Peak VI did not respond to UAA (Table 1) or to UUA, UCA, UGU, UGC, UGG, CGA, AGA, or GGA (Table 2), demonstrating that the response to UGA does not involve the recognition of two out of three bases in the trinucleotide, and that the response is specific for UGA.

Codon recognition studies in other species: Seryl-tRNA of rabbit and chicken liver was fractionated for codon recognition studies by the procedure described above. Only the peak of seryl-tRNA that eluted in 1 M sodium chloride was studied. The seryl-tRNA from rabbit liver responded markedly to the codon UGA and only slightly or not at all to the other codons investigated (Table 3). The chicken liver seryl-tRNA responded to UGA, UCU, and UCC (Table 3). It would appear from the studies on Peak VI of beef brain (see

TABLE 3. Codon responses of rabbit and of chicken liver seryl-tRNA.

Codon	Rabbit liver Δ pmol bound*	Chicken liver Δ pmol bound*
UGA	0.266	0.079
UCU	0.005	0.035
UCC	0.005	0.035
UCA	0.006	0.015
UCG	0.004	0.011
AGU	0.004	0.011
AGC	-0.010	0.013
UAA	-0.008	0.002
UAG	0.005	0.015
None†	(0.076)	(0.045)

Codon responses of rabbit and of chicken liver seryl-tRNA. Assay conditions as in Table 1. 1.020 pmol of rabbit liver [³H]seryl-tRNA (0.19 A_{260} units) and 0.579 pmol of chicken liver [³H]seryl-tRNA (0.34 A_{260} units) were employed in assays. Only the seryl-tRNA which eluted from the reversed-phase chromatographic column in 1 M sodium chloride was assayed.

* Amount of seryl-tRNA bound to ribosomes in presence of codon minus the amount bound in absence of codon.

† Amount of seryl-tRNA bound to ribosomes in absence of codon.

Tables 1 and 2) that a separate species of seryl-tRNA, which recognizes UCU, is present in this fraction. The most pronounced response, however, was to UGA. Clearly, a species of seryl-tRNA is present in chicken and rabbit liver that responds to UGA.

Deacylation and identification of serine: Each peak of seryl-tRNA in liver and in brain (Fig. 3A and B, respectively) was deacylated in ammonium formate,

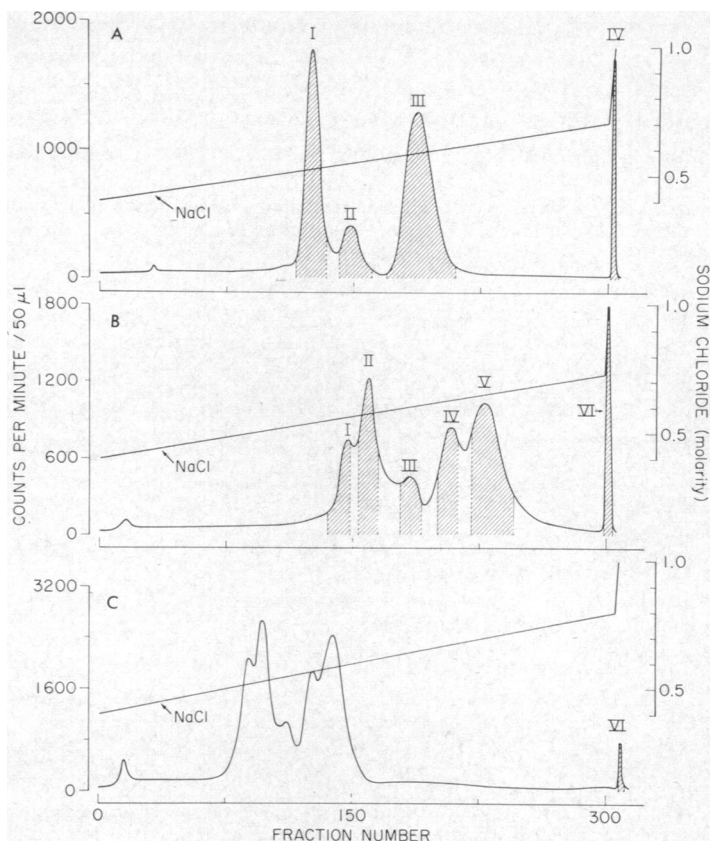


FIG. 3. Fractionation of beef liver and brain seryl-tRNA for codon recognition studies. (A): Liver tRNA was acylated with liver amino acyl-tRNA synthetases and [^3H]serine. Column conditions were the same as those given in the legend of Fig. 1. Radioactivity was detected in 50- μl aliquots taken from 10-ml fractions, that were pooled as shown in the figure and prepared for codon recognition and deacylation studies. (B): Brain tRNA was acylated with liver amino acyl-tRNA synthetases and [^3H]serine. (C) Brain tRNA was acylated with liver amino acyl-tRNA synthetases and [^3H]serine. Only the peak which eluted in 1 M NaCl was used in the codon recognition studies.

pH 10.5, at 37°C for 1 hr, and the free amino acid was determined to be serine by paper chromatography and electrophoresis.¹ In these studies, conditions were optimal for the formation of seryl-tRNA, but may not have been optimal for the synthesis of derivatives of the serine moiety on seryl-tRNA (see discussion).

Discussion. The seryl-tRNA of beef, rabbit, and chicken liver and of beef brain, which eluted from reversed phase chromatographic columns in 1 M sodium chloride, responded to the codon UGA. However, it is difficult to ascertain the role of UGA, or of the seryl-tRNA (seryl-tRNA_{UGA}) which recognizes this codon, in higher organisms. In bacteria, UGA and a protein, release factor R2, are involved in termination of protein synthesis.^{28,29} If a release factor and UGA are involved in termination in higher organisms, then it would seem that seryl-tRNA_{UGA} is a suppressor tRNA. Suppression of UGA has been described in

bacteria.³³ If this codon is not involved in termination in higher organisms, then UGA may be a codeword for seryl-tRNA. Studies to elucidate the role of seryl-tRNA_{UGA} in higher organisms, as well as the possibility that the seryl moiety may undergo further modification to *O*-phosphorylseryl-tRNA,³⁴ to *N*-acetylseryl-tRNA,³⁵ or to *N*-formylseryl-tRNA³⁶ are presently being investigated.

We thank Dr. M. Nirenberg for the gift of the trinucleoside diphosphates and Drs. M. Nirenberg, R. Gallo, and A. Peacock for suggestions and helpful criticisms during preparation of this manuscript. We also gratefully acknowledge the excellent technical assistance of Mrs. M. Caicuts.

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